

## Comparing the demonstration of freedom from *Trichinella* infection of domestic pigs by traditional and risk-based surveillance

M. E. SCHUPPERS<sup>1</sup>\*, C. F. FREY<sup>2</sup>, B. GOTTSTEIN<sup>2</sup>, K. D. C. STÄRK<sup>3</sup>,  
U. KIHM<sup>1</sup> AND G. REGULA<sup>4</sup>

<sup>1</sup> SAFOSO, Bern, Switzerland

<sup>2</sup> Institute of Parasitology, Vetsuisse Faculty, University of Bern, Bern, Switzerland

<sup>3</sup> Royal Veterinary College, Department of Veterinary Clinical Science, Hertfordshire, UK

<sup>4</sup> Veterinary Public Health Institute, University of Bern, Bern, Switzerland

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### SUMMARY

Traditionally, the routine artificial digestion test is applied to assess the presence of *Trichinella* larvae in pigs. However, this diagnostic method has a low sensitivity compared to serological tests. The results from artificial digestion tests in Switzerland were evaluated over a time period of 15 years to determine by when freedom from infection based on these data could be confirmed. Freedom was defined as a 95% probability that the prevalence of infection was below 0·0001%. Freedom was demonstrated after 12 years at the latest. A new risk-based surveillance approach was then developed based on serology. Risk-based surveillance was also assessed over 15 years, starting in 2010. It was shown that by using this design, the sample size could be reduced by at least a factor of 4 when compared with the traditional testing regimen, without lowering the level of confidence in the *Trichinella*-free status of the pig population.

**Key words:** Freedom from infection, pigs, risk-based surveillance, *Trichinella* spp.

### INTRODUCTION

Nematodes of the genus *Trichinella* are the causative agents of trichinellosis, a zoonotic disease with clinical symptoms in humans ranging from mild to fatal. *Trichinella* spp. also occur in many carnivorous and omnivorous animal species, but animal infections do not lead to clinical signs [1, 2]. Transmission of infection occurs via the intake of meat containing infective larvae [3, 4]. Appropriate heat or freezing treatment are effective to inactivate larvae [5], and therefore human infections are caused by the

consumption of raw or undercooked meat. Wild boar meat, horse meat and pork are the main sources for human infection in Europe [6].

Testing of all slaughtered pigs for the presence of larvae is mandatory in the European Union (EU) and Switzerland to prevent human disease [7]. Despite routine testing at pig slaughter in Switzerland since 2001, no larvae have ever been detected [8]. A recent study also failed to detect anti-*Trichinella* antibodies in domestic pigs [9]. Presence of antibodies without direct detection of the parasite would be an indicator for the presence of low-grade *Trichinella* infections that are not detectable by routine artificial digestion.

EU Regulation 2075/2005 requires that 1 g (finisher pigs) or 2 g (adult pigs) of diaphragm tissue per

\* Author for correspondence: M. E. Schuppers, SAFOSO, Bremgartenstrasse 109A, 3012 Bern, Switzerland.  
(Email: manon.schuppers@safoso.ch)

pig are tested using the routine artificial digestion method during meat inspection. The sensitivity of this method depends on the larval density of the positive samples. Above a larval density of 3–5 larvae per gram (LPG), a sensitivity of 100% was achieved, but below 1 LPG the sensitivity dropped to 40% [10]. Because ~15–20% of naturally infected pigs harboured larval densities of <1 LPG [11], infected pigs may not be detected reliably by this method. Despite the large financial efforts involved in testing of all slaughtered pigs during meat inspection, this surveillance is not adequate to prevent human consumption of pork containing low larval densities. However, if surveillance continues over several years without detecting any infected pigs, these surveillance data can be used to demonstrate that the domestic pig population of a country is free from *Trichinella* infection [12, 13].

Instead of applying the routine artificial digestion method to all pigs during meat inspection, a risk-based surveillance programme could be developed that targets high-risk pigs and uses a diagnostic test protocol with a high sensitivity. Targeted sampling of high-risk pigs increases the confidence that infection is truly absent when all samples test negative, whereas a diagnostic test system with a high sensitivity increases the probability of detecting infection if present. Such a risk-based surveillance programme should provide at least an equivalent level of consumer protection as the current meat inspection programme.

The probability of infection of a pig depends on age and housing conditions. In older pigs this probability is higher due to the cumulative effect of longer lives [11]. Housing conditions determine access to potentially infected wildlife (carrion) and feeding of slaughter and kitchen waste, both of which are important routes of infection [3, 14]. Swiss pig production meets high hygiene standards, thus reducing the importance of feeding of waste materials, but *T. britovi* is known to occur in Swiss wildlife [15, 16]. Domestic pigs with outdoor access therefore have a higher probability of being exposed to *Trichinella* spp. than pigs entirely raised indoors.

The first goal of this study was to evaluate the probability that the Swiss slaughter pig population is truly free from *Trichinella* larvae based on the data from the current meat inspection programme, and to model the future probability of freedom if this surveillance is continued in its current format. The second goal was to develop a risk-based surveillance programme for *Trichinella* spp. in domestic pigs that

provides an equivalent probability of freedom from infection in the Swiss pig population.

## MATERIALS AND METHODS

### Target population

The target population for this study consisted of all slaughtered pigs in Switzerland, the unit of surveillance being one slaughtered pig. The time period for analysis was 1 year.

### Model

Disease freedom is usually defined as a certain level of confidence that the true prevalence is below a specified design prevalence [17]. Freedom from *Trichinella* infection of the target population can be demonstrated when all pigs tested within the surveillance programme have negative test results. The achieved probability of freedom depends on the number of tested pigs and the test characteristics of the diagnostic test. The probability of freedom increases when all test results are negative for multiple surveillance time periods. A Bayesian approach [12, 13] was used to calculate the probability of freedom using data from multiple surveillance time periods. The model depends on several parameters:

- the design prevalence,  $P^*$ ;
- the sensitivity of the surveillance system,  $SSe$ ;
- the probability of introduction,  $PIntro$ ;

At the beginning of each time period  $tp$ , a certain prior probability exists that the target population is infected. This probability is reflected by  $PriorPinf_{tp}$ . At the end of  $tp$  it is possible to calculate the posterior probability of freedom  $PostPfree_{tp}$  using Bayes' theorem assuming perfect specificity of the surveillance system [12, 13]:

$$PostPfree_{tp} = \frac{1 - PriorPinf_{tp}}{1 - PriorPinf_{tp} * SSe_{tp}}. \quad (1)$$

Two alternative designs were calculated and compared. In the first design, the surveillance programme was based on the use of the routine artificial digestion test at slaughter. Slaughtered pigs were tested without consideration of their relative risk (RR) of infection, so no risk groups were included in the first design. Data from the routine artificial digestion test were used that were available for the period 2001–2007. Data from 2007 were extrapolated until 2015 to

Table 1. *Scenario tree structure for risk-based serological Trichinella surveillance in domestic pigs in Switzerland, assuming perfect specificity of the surveillance system*

Age	Housing condition	Animal status	ELISA result	Western blot result	Outcome
Finishing pigs	Indoor	Infected	Positive	Positive	Positive
			Negative	Negative	Negative
	Outdoor	Uninfected			Negative
		Infected	Positive	Positive	Positive
	Free-range	Infected	Negative	Negative	Negative
			Positive	Positive	Positive
		Uninfected			Negative
			Infected	Negative	Negative
		Infected	Positive	Positive	Positive
			Negative	Negative	Negative
Adult pigs	Indoor	Uninfected			Negative
		Infected	Positive	Positive	Positive
	Outdoor	Uninfected			Negative
		Infected	Positive	Positive	Positive
	Free-range	Uninfected	Negative	Negative	Negative
			Infected	Negative	Negative
		Infected	Positive	Positive	Positive
			Negative	Negative	Negative
		Uninfected			Negative
			Infected	Negative	Negative

obtain a 15-year surveillance period, assuming the surveillance system would not change from 2008 to 2015, and no positive results would be recorded. This assumption was considered reasonable, because the data from 2007 reflected a full-scale testing programme in Switzerland and the size of the slaughter pig population has remained stable over the last 7 years.

In the second design, a risk-based, serological surveillance programme was considered. An ELISA was used as screening test, and a Western Blot assay (WB) was used as a confirmatory test for any ELISA-positive samples [9, 18]. The target population was divided into different risk groups depending on age and housing conditions, and groups with a higher risk were sampled more intensively than groups with a lower risk. The risk-based surveillance programme was also modelled for a 15-year period starting in 2010, directly following 9 years of surveillance in design 1.

The model was built as a scenario tree with multiple branches (Table 1). First, the total pig population was stratified according to the risk factors age and housing

condition. Then, the probability of infection for a randomly selected pig in each of the different strata was determined. Clustering at herd level was not included in the model, because trichinellosis is not a contagious disease and the mere presence of an infected pig therefore does not increase the probability of infection for nearby pigs.

For infected pigs, the diagnostic test system could either correctly confirm this status (outcome = positive), or fail to detect the infected pig (outcome = negative). The specificity of the surveillance system was considered to be 100%. The assumption of perfect specificity is common for programmes demonstrating freedom [17, 19], because a positive finding after confirmatory investigations would imply the loss of the 'free status' and the surveillance to demonstrate freedom would be replaced by surveillance to regain the 'free status'. Moreover, the specificity of the WB was 100% or very near [18, 20, 21].

The models were created in Microsoft Excel with the add-in @Risk (Palisade Inc., USA). The models were stochastic models with appropriate probability

Table 2. Number of pigs slaughtered and tested for *Trichinella* spp. in Switzerland, 2001–2007

Year	Pigs slaughtered	Pigs tested	Per cent tested	Positive results
2001	2 745 186	404 881	14.7	0
2002	2 729 495	404 674	14.8	0
2003	2 646 905	484 623	18.3	0
2004	2 608 978	488 768	18.7	0
2005	2 712 779	916 791	33.8	0
2006	2 801 133	1 249 091	44.6	0
2007	2 782 708	2 420 008	87.0	0

Source: Federal Veterinary Office, Swiss Zoonoses Reports 2005–2008 (<http://www.bvet.admin.ch/dokumentation/00327/index.html?lang=en>). Accessed 23 July 2009.

distributions as inputs, and were run with 10 000 iterations. A regression analysis was conducted in @Risk to identify the input parameters with the greatest influence on the model outcome (probability of freedom from infection).

### Slaughter pig population

In the period 2001–2007, 2.6–2.8 million pigs were slaughtered annually in Switzerland (Table 2). Routine artificial digestion tests had been implemented voluntarily since 2001 and were made compulsory in 2007 [22], although an exception is made for small-scale slaughterhouses that only market their products locally. The results of the routine artificial digestion tests are presented in Table 2. For the risk-based surveillance programme a slaughter pig population of 2.7 million pigs per year was assumed. The slaughter statistics did not allow differentiation between age categories or housing conditions. Therefore, these data had to be derived from other sources.

In 2006, the adult pig population was estimated at 155 000 animals [23]. Assuming an annual replacement rate of ~40%, around 62 000 adult pigs were slaughtered in 2006, representing 2.3% of the total slaughter pig population. This percentage was similar to the numbers presented for Denmark [19]. The proportion of slaughtered finishing pigs ( $PrP_{\text{finish}}$ ) was thus modelled as  $\text{Pert}(0.97, 0.98, 0.99)$  to allow for small variations in the actual proportions and the proportion of slaughtered adult pigs ( $PrP_{\text{adult}}$ ) as  $1 - PrP_{\text{finish}}$ .

A large proportion of the Swiss pig population is kept in production systems with access to outdoor areas. According to the annual report of the Swiss

Federal Office of Agriculture [24], 61% of all finishing pigs and 58% of all adult pigs have access to outdoor areas. In the majority of cases, these outdoor areas consist of small, confined areas with concrete floors (housing condition: outdoor). Rarely, pigs are kept on pasture under extensive conditions (free-range), but no estimates for the number of pigs in this category were available. Using expert opinion, it was estimated that 2% of all finishing pigs and 1% of all adult pigs fell in the free-range category. The remaining pigs (37% of all finishing pigs and 41% of all adult pigs) were assumed to be produced under intensive conditions without outdoor access (indoor). To account for uncertainty around these point estimates, the proportions of indoor finishing pigs ( $PrP_{\text{finish,in}}$ ) and indoor adult pigs ( $PrP_{\text{adult,in}}$ ) were modelled as  $\text{Pert}(0.32, 0.37, 0.42)$  and  $\text{Pert}(0.36, 0.41, 0.46)$ , respectively. The proportion of outdoor finishing pigs ( $PrP_{\text{finish,out}}$ ) was modelled as  $\text{Pert}(0.56, 0.61, 0.66)$  and of outdoor adult pigs ( $PrP_{\text{adult,out}}$ ) as  $\text{Pert}(0.53, 0.58, 0.63)$ . The proportion of free-range finishing pigs was then calculated as  $1 - (PrP_{\text{finish,in}} + PrP_{\text{finish,out}})$  and of free-range adult pigs as  $1 - (PrP_{\text{adult,in}} + PrP_{\text{adult,out}})$ .

### Design prevalence and effective probability of infection

$P^*$  was set at 0.0001%, as defined by EU Regulation 2075/2005. Although  $P^*$  applied to the whole target population, the effective probability of infection (EPI) differed between the different risk groups. However, the average EPI of all pigs still equalled  $P^*$ .

The EPI for a pig is derived from the RRs associated with the applicable levels of each of the risk factors specified, i.e. age and housing condition. For each risk factor, RR is the risk of infection in its risk category relative to the risk in the lowest risk category for that risk factor. No cases of *Trichinella*-positive pigs have been reported in Switzerland, and also in other Western European countries there is a lack of data to reliably determine the RR of individual pigs in the different risk groups.

The RR of adult pigs in comparison to finishing pigs is derived from the longer lifespan and thus the increased probability of infection at some time during life. Finishing pigs are slaughtered at around age 6 months, and the average breeding sow is slaughtered at around 3.5 years of age (assuming five litters per sow). If the probability of infection during life increased linearly, at slaughter a breeding sow would have a seven times higher probability of having

Table 3. *Relative risks of Trichinella infection associated with age and housing condition in four combinations (schemes), and adjusted prevalence (effective probability of infection) for each risk group separately. Design prevalence for whole population,  $P^* = 0.0001\%$*

Scheme	Risk group	Population proportion	Relative risk	Effective probability of infection
1	Finishing pigs	98.0 %	1	
	Indoor	37.0 %	1	0.000024 %
	Outdoor	61.2 %	5	0.000119 %
	Free-range	1.8 %	25	0.000596 %
	Adult pigs	2.0 %	5	
	Indoor	41.0 %	1	0.000131 %
	Outdoor	58.1 %	5	0.000653 %
	Free-range	0.9 %	25	0.003265 %
2	Finishing pigs	98.0 %	1	
	Indoor	37.0 %	1	0.000011 %
	Outdoor	61.2 %	10	0.000111 %
	Free-range	1.8 %	100	0.001113 %
	Adult pigs	2.0 %	5	
	Indoor	41.0 %	1	0.000065 %
	Outdoor	58.1 %	10	0.000648 %
	Free-range	0.9 %	100	0.006482 %
3	Finishing pigs	98.0 %	1	
	Indoor	37.0 %	1	0.000022 %
	Outdoor	61.2 %	5	0.000109 %
	Free-range	1.8 %	25	0.000545 %
	Adult pigs	2.0 %	10	
	Indoor	41.0 %	1	0.000239 %
	Outdoor	58.1 %	5	0.001195 %
	Free-range	0.9 %	25	0.005976 %
4	Finishing pigs	98.0 %	1	
	Indoor	37.0 %	1	0.000010 %
	Outdoor	61.2 %	10	0.000102 %
	Free-range	1.8 %	100	0.001019 %
	Adult pigs	2.0 %	10	
	Indoor	41.0 %	1	0.000119 %
	Outdoor	58.1 %	10	0.001187 %
	Free-range	0.9 %	100	0.011865 %

acquired an infection than a finishing pig. To account for uncertainty around this assumption, two different RRs for adult pigs in comparison to finishing pigs were used:

$$RR_{\text{adult}} = 5 \quad \text{and} \quad RR_{\text{adult}} = 10.$$

The RR of pigs raised under outdoor or free-range housing conditions in comparison to pigs under indoor housing conditions is determined by the differences in biosecurity of these housing conditions and thus the probability that pigs in these different housing conditions have contact with infected wildlife

or contaminated kitchen or slaughter waste. No estimates for RRs were available, therefore two different increments were selected. First, it was assumed that the RR increased by a factor of 5 between housing conditions ( $RR_{\text{outdoor}} = 5$  and  $RR_{\text{free-range}} = 25$ ). Second, it was assumed that the RR increased by a factor of 10 between housing conditions ( $RR_{\text{outdoor}} = 10$  and  $RR_{\text{free-range}} = 100$ ).

Combining these two risk factors (age and housing condition) into a matrix, four schemes were developed (Table 3). Relative risks were then adjusted to give adjusted risks (ARs), such that the average AR over

the target population was 1 [12, 13]. For age:

$$\sum_{l=1}^L (AR_l * PrP_l) = 1 \quad (2)$$

in which the target population was divided into  $L$  different age categories, and  $PrP_l$  was the proportion of animals in the target population belonging to age group  $l$ . This process was repeated for the risk factor housing condition using the appropriate conditional proportions. Then [12, 13]:

$$EPI_{l,m} = AR_l * AR_{l,m} * P^*. \quad (3)$$

where  $m$  denotes categories of housing condition.

### Diagnostic tests and the sensitivity of the surveillance system

For the routine artificial digestion test, samples of up to 100 pigs can be pooled. It was demonstrated that the sensitivity of a pooled assay with 100 samples did not exceed 40% in case of larval densities <1 LPG [10], a situation that occurs in 15–20% of the pigs infected under field conditions [11]. As a conservative approach for design 1, it was therefore assumed that the sensitivity of the routine artificial digestion test ( $Se_{AD}$ ) was 40% and it was modelled as  $Pert(0.35, 0.40, 0.45)$  [19].

For design 2, an ELISA and WB were used as screening and confirmatory tests, respectively. Various studies evaluated the sensitivity of the ELISA ( $Se_{ELISA}$ ) and reported values from 72.7% to 99.2% [20, 25–28].  $Se_{ELISA}$  was therefore modelled as  $Pert(0.60, 0.95, 1)$ . The WB was recently validated with reported sensitivities of 95.8–98.1% [18, 20, 21]. The sensitivity of the WB ( $Se_{WB}$ ) was therefore modelled as  $Pert(0.90, 0.98, 1)$ .

The  $SSe$  is an estimate of the probability that the surveillance system detects infection in the target population if the prevalence exceeds  $P^*$ .  $SSe$  is calculated as [12, 13]:

$$SSe = 1 - (1 - Se_u)^N \quad (4)$$

in which  $Se_u$  is the probability that a randomly sampled animal (unit) is both infected and detected and  $N$  is the total number of animals in the surveillance system. Equation (4) assumes independence of animals with regard to the probabilities of being infected and detected. In design 1, no risk groups were included and  $Se_u$  was therefore calculated as:

$$Se_u = P^* * Se_{AD}. \quad (5)$$

In design 2, an animal in any of the risk groups can give a positive outcome, so  $Se_u$  was calculated as:

$$Se_u = \sum_{l=1}^L \sum_{m=1}^M PrSSC_{l,m} * EPI_{l,m} * Se_{ELISA} * Se_{WB} \quad (6)$$

in which  $PrSSC_{l,m}$  was the proportion of pigs processed that belonged to the  $l$ th age stratum and the  $m$ th housing condition stratum.

### Probability of introduction

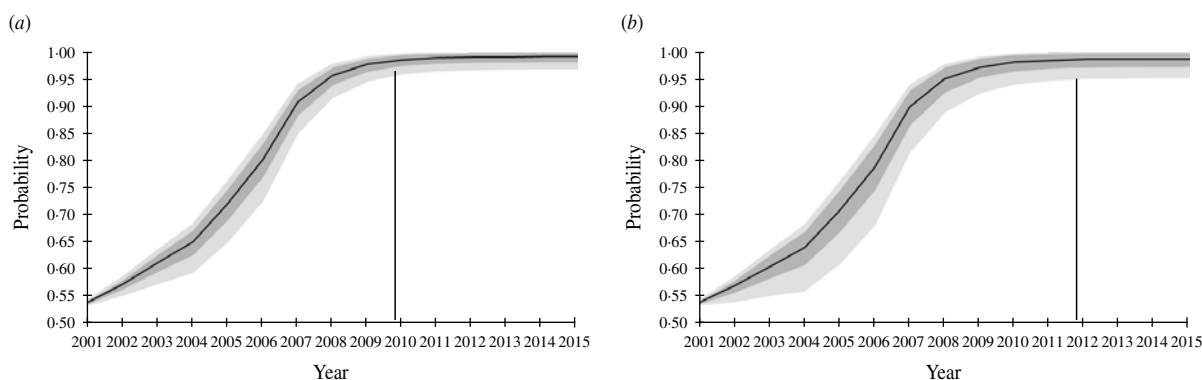
*T. britovi* is present in Swiss wildlife [16], and constitutes a risk for introduction of infection into the domestic pig population. However, no records of infected domestic pigs exist in Switzerland, and  $PIntro$  therefore cannot be derived directly. Alban *et al.* [19] conservatively determined  $PIntro$  for the Danish domestic pig population as 1 divided by the time since the last outbreak, resulting in 1/76. Since this was a conservative estimate, we considered it valid to use a similar  $PIntro$  for the Swiss pig population. We modelled  $PIntro$  as a Beta distribution with 0 introductions in 75 years [Beta(1, 76)], resulting in a median annual  $PIntro$  of 0.91% (95% probability interval 0.03–4.7). Taking into account the higher proportion of pigs having access to outdoor areas in Switzerland and the presence of *T. britovi* in wildlife, we also modelled  $PIntro$  as a Beta distribution with 0 introductions in 50 years [Beta(1, 51)], resulting in a median annual  $PIntro$  of 1.3% (0.05–7.0).

## RESULTS

### Design 1: traditional *Trichinella* surveillance

The  $SSe$  increased gradually from 14.95% in 2001 to 62.02% in 2007, because the sample size increased annually during this period. From 2008–2015 the  $SSe$  remained equal to the  $SSe$  in 2007, because the number of pigs tested was kept constant. The  $PriorPinf_{2001}$  was set at 50%, because no other information was available. Depending on the selected  $PIntro$ , Switzerland could demonstrate freedom from *Trichinella* infection in domestic pigs with 95% confidence by the end of 2010 or 2012 (Fig. 1).

The input parameters  $Se_{AD}$  and  $PIntro$  had the largest influence on the model, although their relative importance changed over time. For example, when  $PIntro = \text{Beta}(1, 76)$ , the regression coefficients of  $Se_{AD}$  and  $PIntro$  changed from 0.64 and  $-0.77$ , respectively after year 2 to 0.12 and  $-0.99$ , respectively after



**Fig. 1.** Probability of freedom from *Trichinella* spp. infection of the Swiss slaughter pig population at a design prevalence of 0.0001 % achieved at the end of each surveillance year using routine artificial digestion without considering risk groups in the pig population. Vertical line indicates year at which end the probability of freedom exceeds 95 %, as expressed conservatively by the lower limit of the 95 % confidence interval. Black line represents mean. Dark grey area,  $\pm 1$  standard deviation; light grey area, 95 % confidence interval. (a) Probability of introduction ( $P_{Intro} = \text{Beta}(1, 76)$ ); (b)  $P_{Intro} = \text{Beta}(1, 51)$ .

year 15. Regression coefficients were very similar when  $P_{Intro} = \text{Beta}(1, 51)$ .

## Design 2: risk-based *Trichinella* surveillance

In risk-based surveillance, freedom from infection must also be demonstrated with at least 95 % probability. The  $PriorPinf_{2010}$  (the year in which the risk-based surveillance programme started) was calculated using the  $PostPinf_{2009}$  of design 1. This was considered appropriate, because the risk-based surveillance programme started immediately after the completion of the traditional surveillance in 2009. The sampling was targeted towards the higher risk groups, and included almost all adult pigs, almost all free-ranging finishing pigs, a large number of outdoor finishing pigs and a small number of indoor finishing pigs. The minimum sample size was determined by increasing the sample size by steps of 10 000 samples until freedom from infection was demonstrated (Table 4). For  $P_{Intro} = \text{Beta}(1, 76)$ , the required sample sizes ranged from 120 000 (scheme 4) to 360 000 (scheme 1). For  $P_{Intro} = \text{Beta}(1, 51)$ , the required sample sizes ranged from 260 000 (scheme 4) to 620 000 (scheme 1). Figure 2 shows the probability of freedom from infection achieved by the risk-based surveillance programme from 2010 to 2024 under scheme 1.

The  $SSe$  differed for each of the four schemes due to different sample sizes, and was also influenced indirectly by  $P_{Intro}$ , because a higher  $P_{Intro}$  resulted in higher sample sizes. After the required sample sizes had been established, the  $SSe$  was determined. For  $P_{Intro} = \text{Beta}(1, 76)$ , the median  $SSe$  of schemes 1–4 varied between 51.3–52.4 %. For  $P_{Intro} = \text{Beta}(1, 51)$ ,

the median  $SSe$  of schemes 1–4 varied between 61.1 and 61.3 %.

After 1 year of surveillance, the model was mainly influenced by four input parameters. For  $P_{Intro} = (1, 76)$ , in scheme 1 the regression coefficients were  $P_{Intro\_design2} = -0.72$ ,  $P_{Intro\_design1} = -0.60$ ,  $Se_{AD} = 0.29$  and  $Se_{ELISA} = 0.10$ . After 15 years, two main input parameters remained:  $P_{Intro\_design2} = -0.98$  and  $Se_{ELISA} = 0.11$ . Regression coefficients were very similar for the other schemes.

## DISCUSSION

This study demonstrated that surveillance by routine artificial digestion test is not capable of demonstrating freedom from *Trichinella* infection in the domestic pig population at the desired level of confidence based on data from a single year in Switzerland. To achieve this, a much larger slaughter pig population would be required than is available in Switzerland. Freedom from *Trichinella* infection by traditional surveillance can only be demonstrated when historical data are incorporated. The method developed by Martin *et al.* [12, 13] allowed this, by assuming that the posterior probability of freedom achieved in year  $t - 1$  could be used to derive the prior probability of freedom in year  $t$ . However, even when historical data were incorporated, freedom from infection could no longer be demonstrated when the sample size was reduced to 1 million pigs per year (data not shown). Therefore, Switzerland would need to continue testing almost all slaughtered pigs at slaughter if routine meat inspection alone was used to demonstrate freedom from infection.

Table 4. Minimum required sample size to demonstrate freedom from *Trichinella* infection of the Swiss domestic pig population with at least 95 % confidence after 15 years of negative risk-based serological surveillance

Risk group (scheme*)	$P_{Intro} = \text{Beta}(1, 76)$				$P_{Intro} = \text{Beta}(1, 51)$			
	1	2	3	4	1	2	3	4
Finishing pigs	306 000	119 000	157 500	66 000	573 500	358 750	441 000	208 000
Indoor	15 300	5950	7875	3300	28 674	17 937	22 050	10 400
Outdoor	244 800	65 450	102 375	29 700	501 813	295 969	374 850	150 800
Free-range	45 900	47 600	47 250	33 000	43 013	44 844	44 100	46 800
Adult pigs	54 000	51 000	52 500	54 000	46 500	51 250	49 000	52 000
Indoor	22 140	20 910	21 525	22 140	19 065	21 012	20 090	21 320
Outdoor	31 320	29 580	30 450	31 320	26 970	29 725	28 420	30 160
Free-range	540	510	525	540	465	513	490	520
Total	360 000	170 000	210 000	120 000	620 000	410 000	490 000	260 000

$P_{Intro}$ , Probability of introduction.

\* Schemes 1–4 each have a different combination of relative risks for the risk factor age (finishing pigs vs. adult pigs) and housing conditions (indoor vs. outdoor vs. free range).

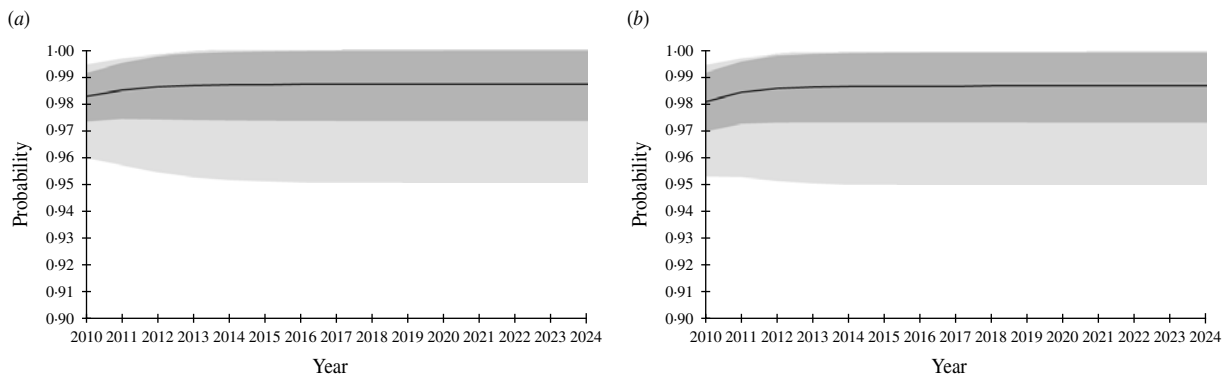


Fig. 2. Probability of freedom from *Trichinella* spp. infection of the Swiss slaughter pig population at a design prevalence of 0.0001 % achieved at the end of each surveillance year using ELISA and Western Blot assay and considering risk groups in the pig population. Black line represents mean. Dark grey area,  $\pm 1$  standard deviation; light grey area, 95 % confidence interval. (a) Probability of introduction ( $P_{Intro}$ ) =  $\text{Beta}(1, 76)$ ; (b)  $P_{Intro}$  =  $\text{Beta}(1, 51)$ .

The sample size could be reduced significantly when serological tests were used and the different risk groups within the pig population were taken into account. Depending on the scheme selected, the annual sample size was reduced by at least a factor of 4 without a loss in the probability of freedom from infection. Further, freedom from infection was already demonstrated after 1 year of risk-based serological surveillance.

Alban *et al.* [19] developed a risk-based surveillance model for *Trichinella* spp. in domestic pigs in Denmark. In this model all adult pigs and all finishing pigs with outdoor access were sampled, whereas finishing pigs from indoor housing systems were not sampled. However, this model used the routine artificial digestion test instead of serology. Serology has

two advantages over the routine artificial digestion test. First, especially with low larval densities the diagnostic sensitivity of ELISA and WB is higher than of routine artificial digestion [10, 20, 25–28]. Second, the number of larvae triggering a detectable antibody response is much lower than the number of larvae that can be detected reliably by routine artificial digestion test [29], leading to a higher analytical sensitivity of serology. Thus, the probability of detecting low-grade infections in pigs increases when serology is used, which additionally supports claims of freedom from infection when all samples are negative.

In the present calculations, a positive outcome was defined as detection of antibodies by both ELISA and WB. Detection of larvae was not included, which is usually considered a reference for determining the



infection status of a pig [5, 30]. However, presence of antibodies indicates that the tested pig has previously been in contact with *Trichinella* spp. False-positive results of the ELISA were excluded by the use of a WB. The combination of both tests was previously shown to have a specificity of at least 99·8–99·9% [18, 20]. In case antibodies were demonstrated by WB, investigations should be initiated on the farm of origin to assess the opportunities for exposure of pigs to *Trichinella* spp.

The sensitivity analysis showed that *PIntro* was the most important input variable for the model. Very limited data were available to estimate *PIntro*. The first approach was to use a similar value as used by Alban *et al.* [19], who already discussed that this value was a conservative estimate. However, the situation in Denmark is different from Switzerland. *T. britovi* is known to occur regularly in Swiss wildlife [15, 16], whereas *Trichinella* spp. is rare in Danish wildlife [31]. Moreover, outdoor housing of pigs is much more common in Switzerland than in Denmark [19, 24]. Therefore, in a second approach an even more conservative *PIntro* was used to take these two differences into account. Further, the sampling in the risk-based surveillance model was heavily targeted towards pigs in the higher risk groups. Despite the increased *PIntro*, freedom from infection could still be demonstrated in the Swiss domestic pig population.

There are very few data about the RRs of pigs acquiring a *Trichinella* infection. It is generally accepted that pigs with outdoor access as well as adult pigs have a higher probability of infection, but this probability was never quantified. Ribicich *et al.* [32] determined that *Trichinella* infections occurred in pigs raised outdoor but not in pigs raised in confinement or partial confinement, however a RR could not be determined. In other studies infections were also detected more frequently in pigs with outdoor access than in pigs in indoor housing systems [33, 34]; however, RRs were not calculated. Alban *et al.* [19] arbitrarily defined four scenarios with different RRs for the high-risk group, ranging from 5·5 to 69. In this study four different schemes for the RR were also used to compensate for the uncertainty around the estimates. Scheme 1 was considered to be the most conservative scheme, because the RRs were minimal. This scheme therefore also leads to the highest required sample sizes.

The ability to identify and trace pigs of the different risk groups clearly is a crucial element for the successful implementation of a risk-based surveillance

system. Currently, such identification and traceability is only possible in Switzerland with an unjustifiably high input of resources. Production labels (e.g. organic production) are poor indicators for the actual pig housing conditions, because farmers may voluntarily exceed the minimum label requirements. Improvement of the pig identification system should be considered before a change to a risk-based surveillance for *Trichinella* spp. is feasible in Switzerland.

In conclusion, this study demonstrated that risk-based serological *Trichinella* surveillance is able to achieve a probability of freedom from infection equivalent to routine artificial digestion, while the required sample size can be reduced by at least a factor of 4.

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## DECLARATION OF INTEREST

None.

## REFERENCES

1. Pozio E. The broad spectrum of *Trichinella* hosts: from cold- to warm-blooded animals. *Veterinary Parasitology* 2005; **132**: 3–11.
2. Kociecka W. Trichinellosis: human disease, diagnosis and treatment. *Veterinary Parasitology* 2000; **93**: 365–383.
3. Pozio E. New patterns of *Trichinella* infection. *Veterinary Parasitology* 2001; **98**: 133–148.
4. Murrell KD, Pozio E. Trichinellosis: the zoonosis that won't go quietly. *International Journal for Parasitology* 2000; **30**: 1339–1349.
5. Gajadhar AA, *et al.* *Trichinella* diagnostics and control: Mandatory and best practices for ensuring food safety. *Veterinary Parasitology* 2009; **159**: 197–205.
6. European Commission. Opinion of the Scientific Committee on Veterinary Measures relating to Public Health on 'Trichinellosis, epidemiology, methods of detection and *Trichinella*-free pig production', 2001 ([http://ec.europa.eu/food/fs/sc/scv/outcome\\_en.html](http://ec.europa.eu/food/fs/sc/scv/outcome_en.html)). Accessed 23 July 2009.
7. European Commission. Commission Regulation 2075/2005 of 5 December 2005 laying down specific rules on official controls for *Trichinella* in meat (<http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2005:338:0060:0082:EN:PDF>). Accessed 29 September 2009.

8. **Federal Veterinary Office.** Swiss zoonosis report, 2008 (<http://www.bvet.admin.ch/dokumentation/00327/02466/02762/index.html?lang=en>). Accessed 23 July 2009.
9. **Schuppers ME, et al.** A study to demonstrate freedom from *Trichinella* spp. in domestic pigs in Switzerland. *Zoonoses and Public Health* 2009. Published online: 8 December 2009. doi:10.1111/j.1863-2378.2009.01299.x.
10. **Forbes LB, Gajadhar AA.** A validated *Trichinella* digestion assay and an associated sampling and quality assurance system for use in testing pork and horse meat. *Journal of Food Protection* 1999; **62**: 1308–1313.
11. **Pozio E, Rossi P.** Guidelines for the identification and development of sampling methods and design of suitable protocols for monitoring of *Trichinella* infection in indicator species. *Annali dell'Istituto Superiore di Sanita* 2008; **44**: 200–204.
12. **Martin PA, Cameron AR, Greiner M.** Demonstrating freedom from disease using multiple complex data sources 1: a new methodology based on scenario trees. *Preventive Veterinary Medicine* 2007; **79**: 71–97.
13. **Martin PA, et al.** Demonstrating freedom from disease using multiple complex data sources 2: case study-classical swine fever in Denmark. *Preventive Veterinary Medicine* 2007; **79**: 98–115.
14. **Pozio E.** Factors affecting the flow among domestic, synanthropic and sylvatic cycles of *Trichinella*. *Veterinary Parasitology* 2000; **93**: 241–262.
15. **Gottstein B, et al.** Epidemiological investigation of trichinellosis in Switzerland. *Veterinary Parasitology* 1997; **72**: 201–207.
16. **Frey CF, et al.** Assessment of the prevalence of *Trichinella* spp. in red foxes and Eurasian lynxes from Switzerland. *Veterinary Parasitology* 2009; **159**: 295–299.
17. **More SJ, et al.** Defining output-based standards to achieve and maintain tuberculosis freedom in farmed deer, with reference to member states of the European Union. *Preventive Veterinary Medicine* 2009; **90**: 254–267.
18. **Frey CF, et al.** Validation of a Western Blot for the detection of anti-*Trichinella* spp. antibodies in domestic pigs. *Parasitology Research* 2009; **104**: 1269–1277.
19. **Alban L, et al.** Towards a risk-based surveillance for *Trichinella* spp. in Danish pig production. *Preventive Veterinary Medicine* 2008; **87**: 340–357.
20. **Frey CF, et al.** Evaluation of a new commercial enzyme-linked immunosorbent assay for the detection of porcine antibodies against *Trichinella* spp. *Journal of Veterinary Diagnostic Investigation* 2009; **21**: 692–697.
21. **Nöckler K, et al.** Evaluation of a Western Blot and ELISA for the detection of anti-*Trichinella*-IgG in pig sera. *Veterinary Parasitology* 2009; **163**: 341–347.
22. **Anon.** Verordnung über das Schlachten und die Fleischkontrolle. Nr 817.190, 2005. [http://www.admin.ch/ch/d/sr/c817\\_190.html](http://www.admin.ch/ch/d/sr/c817_190.html). Accessed 29 September 2009.
23. **Proviande.** Der Fleischmarkt im Überblick 2007 (<http://www.schweizerfleisch.ch/medium.php?l=1&id=214771>). Accessed 23 July 2009.
24. **Federal Office for Agriculture.** Agrarbericht 2007 (<http://www.blw.admin.ch/dokumentation/00018/00498/index.html?lang=de>). Accessed 3 December 2008.
25. **Murrell KD, et al.** Field evaluation of the enzyme-linked immunosorbent assay for swine trichinellosis: efficacy of the excretory-secretory antigen. *American Journal of Veterinary Research* 1986; **47**: 1046–1049.
26. **Oliver DG, et al.** Field evaluation of an enzyme immunoassay for detection of hogs in a high volume North Carolina abattoir. In: Tanner CE, ed. *Trichinellosis*. Madrid, Spain: Consejo Superior de Investigaciones Cientificas Press, 1989, pp. 439–444.
27. **van der Leek ML, et al.** Evaluation of an enzyme-linked immunosorbent assay for diagnosis of trichinellosis in swine. *American Journal of Veterinary Research* 1992; **53**: 877–882.
28. **Nöckler K, et al.** Influence of methods for *Trichinella* detection in pigs from endemic and non-endemic European region. *Journal of Veterinary Medicine, Series B: Infectious Diseases and Veterinary Public Health* 2004; **51**: 297–301.
29. **Gamble HR, et al.** Diagnosis of swine trichinosis by enzyme-linked immunosorbent assay (ELISA) using an excretory-secretory antigen. *Veterinary Parasitology* 1983; **13**: 349–361.
30. **Gamble HR, et al.** International Commission on Trichinellosis: recommendations on methods for the control of *Trichinella* in domestic and wild animals intended for human consumption. *Veterinary Parasitology* 2000; **93**: 393–408.
31. **Enemark HL, et al.** Screening for infection of *Trichinella* in red fox (*Vulpes vulpes*) in Denmark. *Veterinary Parasitology* 2000; **88**: 229–237.
32. **Ribicich M, et al.** Evaluation of the risk of transmission of *Trichinella* in pork production systems in Argentina. *Veterinary Parasitology* 2009; **159**: 350–353.
33. **van der Giessen J, et al.** Seroprevalence of *Trichinella spiralis* and *Toxoplasma gondii* in pigs from different housing systems in The Netherlands. *Veterinary Parasitology* 2007; **148**: 371–374.
34. **Gebreyes WA, et al.** Seroprevalence of *Trichinella*, *Toxoplasma*, and *Salmonella* in antimicrobial-free and conventional swine production systems. *Foodborne Pathogens and Disease* 2008; **5**: 199–203.